Journal of Pharmacological Sciences 129 (2015) 38–42

HOSTED BY

ELSEVIER

Contents lists available at ScienceDirect

Journal of Pharmacological Sciences

journal homepage: www.elsevier.com/locate/jphs

Full paper

Pharmacological characterization of the involvement of protein kinase C in oscillatory and non-oscillatory calcium increases in astrocytes

Mitsuhiro Morita [a, b, *], Akira Nakane [b], Shohei Maekawa [a], Yoshihisa Kudo [b]

[a] Department of Biology, Kobe University Graduate School of Science, Kobe, 657-8501, Japan
[b] School of Life Science, Tokyo University of Pharmacy and Life Science, Tokyo, 192-0392, Japan

ARTICLE INFO

Article history:
Received 23 February 2015
Received in revised form
9 August 2015
Accepted 11 August 2015
Available online 22 August 2015

Keywords:
Astrocyte
Calcium oscillation
Protein kinase C
Glutamate
Histamine

ABSTRACT

Evidence increasingly shows that astrocytes play a pivotal role in brain physiology and pathology via calcium dependent processes, thus the characterization of the calcium dynamics in astrocytes is of growing importance. We have previously reported that the epidermal growth factor and basic fibroblast growth factor up-regulate the oscillation of the calcium releases that are induced by stimuli, including glutamate in cultured astrocytes. This calcium oscillation is assumed to involve protein kinase C (PKC), which is activated together with the calcium releases as a consequence of inositol phospholipid hydrolysis. In the present study, this issue has been investigated pharmacologically by using astrocytes cultured with and without the growth factors. The pharmacological activation of PKC largely reduced the glutamate-induced oscillatory and non-oscillatory calcium increases. Meanwhile, PKC inhibitors increased the total amounts of both calcium increases without affecting the peak amplitudes and converted the calcium oscillations to non-oscillatory sustained calcium increases by abolishing the falling phases of the repetitive calcium increases. Furthermore, the pharmacological effects were consistent between both glutamate- and histamine-induced calcium oscillations. These results suggest that PKC up-regulates the removal of cytosolic calcium in astrocytes, and this up-regulation is essential for calcium oscillation in astrocytes cultured with growth factors.

1. Introduction

Evidence increasingly indicates that calcium dependent processes in astrocytes play a pivotal role in brain physiology and pathology. Intracellular calcium in astrocytes increases spontaneously or in response to diverse stimuli, including neurotransmitters, hormones, and lipid metabolites (1–3), and triggers the releases of substances that affect neuronal activities or cerebral circulations (4,5). Calcium increases also alter the structure and gene expression of astrocytes (6). The spatiotemporal pattern of calcium increases in astrocytes is altered by cytokine treatments *in vitro* and by pathological conditions *in vivo*, including epilepsy and dementia (7–9), suggesting that the calcium dynamics in astrocyte also participate in pathological processes in the brain. Therefore, characterizing the mechanisms underlying the calcium increase patterns of astrocytes is an important issue for understanding brain functions and diseases.

We have previously demonstrated that epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) upregulate the oscillation of the calcium releases that are induced by stimuli including glutamate in astrocytes (7). In virtue of this finding, we have established a defined medium that is suitable to study the astrocyte calcium oscillation, which is induced in a limited population of the astrocytes cultured in a conventional serum-containing culture media. Most of the astrocytes cultured in a defined medium containing growth factors release calcium in an oscillatory manner, whereas the calcium release is largely non-oscillatory if the astrocytes are cultured without the growth factors. This culture model has allowed us to demonstrate that the astrocyte calcium oscillation is regulated by the mitogen-activated protein kinase and the expression of sarco-endoplasmic reticulum calcium ATPase (SERCA) (10).

In the present study, we have pharmacologically characterized the involvement of protein kinase C (PKC) in the calcium dynamics in astrocytes. PKC is activated by diacylglycerol and calcium, both of which increase following receptor-induced inositol phospholipid

* Corresponding author. Department of Biology, Kobe University Graduate School of Science, 1-1 Rokkodai Nada, Kobe, Hyogo, 657-8501, Japan. Tel./fax: +81 78 803 6636.
E-mail address: mmorita@boar.kobe-u.ac.jp (M. Morita).
Peer review under responsibility of Japanese Pharmacological Society.

http://dx.doi.org/10.1016/j.jphs.2015.08.004

hydrolysis. In addition, some astrocyte functions, which are elicited by the activation of calcium-inducing metabotropic glutamate receptors, are mediated by PKC (11,12). Thus, PKC is likely to be active during glutamate-induced calcium increases in astrocytes. A large number of receptors and cellular machineries that are involved in calcium dynamics have been reported as substrates of PKC (13). Therefore, it is assumed that PKC influences the spatiotemporal pattern of calcium increases in astrocytes. To reveal the contribution of PKC to the oscillatory and non-oscillatory patterns of calcium increases in astrocytes, the present study has examined the effects of pharmacological modifications of PKC on calcium increases in astrocyte cultured in the presence or absence of growth factors. Our results demonstrate that PKC is actively involved in calcium dynamics in astrocytes.

2. Materials and methods

2.1. Cell culture

All of the animal experiments were approved by the Institutional Animal Care and Use Committee of Tokyo University of Pharmacy and Life Sciences or Kobe University (IACUC). Astrocytes were isolated from the cerebral cortices of postnatal day 1 Wistar rats, as described previously (14). Briefly, brain cells were prepared by trypsinization and cultured for 12 days in basal Eagle's medium containing 10% fetal calf serum (FCS). The resulting mixed glial culture was shaken at 260 rpm for 18 h to remove non-astrocytic cells. The adherent cells were subcultured by trypsinization and seeded in Dulbecco's Modified Eagle's medium (DMEM) containing 25 mM HEPES, pH 7.4, and 10% FCS. After 48 h, the medium was changed to DMEM containing 25 mM HEPES, pH 7.4, 1 mM pyruvate, 2 mM glutamine, 50 μg/mL human apo-transferrin (Invitrogen, Carlsbad, CA), 10 ng/mL D-biotin, 5.2 ng/mL sodium selenite, 1.5 μg/mL bovine fibronectin (Invitrogen), 0.5 μg/mL heparan sulfate, 5 μg/mL of insulin (Invitrogen), 10 ng/mL EGF (Invitrogen), and 5 ng/mL bFGF (Invitrogen). The cells were cultured for 48–96 h before the calcium imaging experiments, whose results can be seen in Figs. 1 and 3. Astrocytes were cultured without EGF and bFGF for the experiments that are illustrated in Fig. 2.

2.2. Calcium imaging

The method that we used for the calcium imaging of the astrocytes in the defined medium, included quantification of calcium fluorescence values and setting region of interest, has been described in detail in our previous paper (15). The cells were loaded for 45 min at 30 °C with 7.5 μM Fura2AM (Dojin, Kumamoto, Japan) in basal salt solution (BSS), consisting of: 129 mM NaCl, 4 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 4.2 mM glucose, and 10 mM HEPES adjusted to pH 7.4 with NaOH. In all of the steps after loading, 100 μM sulfinpyrazone was added to the BSS. The durations of pretreatment with phorbol 12-myristate 13- acetate (PMA), Go6976, Go6983, and GF109203X (GFX) were 5 min, 60 min, 60 min, and 10 min, respectively. Calcium increases were induced by adding agonists by perfusing at 1.5 ml/min. Calcium imaging was performed using an IX70 inverted microscope, a UApo/340 40x/1.15w objective, and an OSP-EXA filter exchanger (all from Olympus, Tokyo, Japan) equipped with a C6790 CCD camera (Hamamatsu Photonics, Hamamatsu, Japan). Fluorescence images were acquired and the R340/380 ratios were calculated using AQUACOSMOS software (Hamamatsu Photonics). Each quantitative analysis of a calcium increase represents at least four imaging experiments using two series of cultures from two different cell preparations.

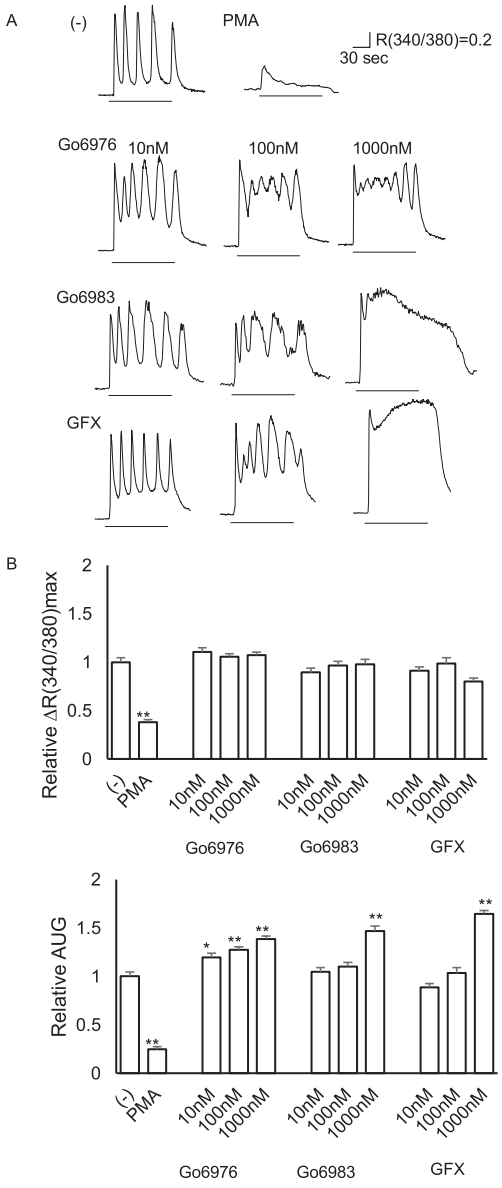

Fig. 1. The effects of pharmacological modifications of PKC on glutamate-induced calcium oscillations in astrocytes cultured with growth factors. (A) Representative calcium increases. Cells were pretreated with 300 nM PMA or 10–1000 nM PKC inhibitors (Go6972, Go6983 or GFX) and then stimulated with 30 μM glutamate, as indicated by the underlines. (B) Quantitative comparisons of calcium increases by relative peak amplitude; ΔR(340/380)max and relative area under the graph; AUG. Mean ± S.E.M. *$p < 0.05$, **$p < 0.01$, Tukey's multiple comparison method with control ($n = 28$ cells).

2.3. Chemicals

Go6976 and Go6983 were from Calbiochem (Cambridge, MA, USA). GF109203X (GFX) was obtained from Enzo Life Sciences (Farmingdale, NY, USA). Unless otherwise indicated, the chemicals were supplied by Sigma (St. Louis, MO, USA).

3. Results

3.1. Glutamate-induced calcium oscillation

The involvement of PKC in the glutamate-induced calcium oscillations in astrocytes cultured with growth factors has been investigated pharmacologically. As shown in Fig. 1, the PKC

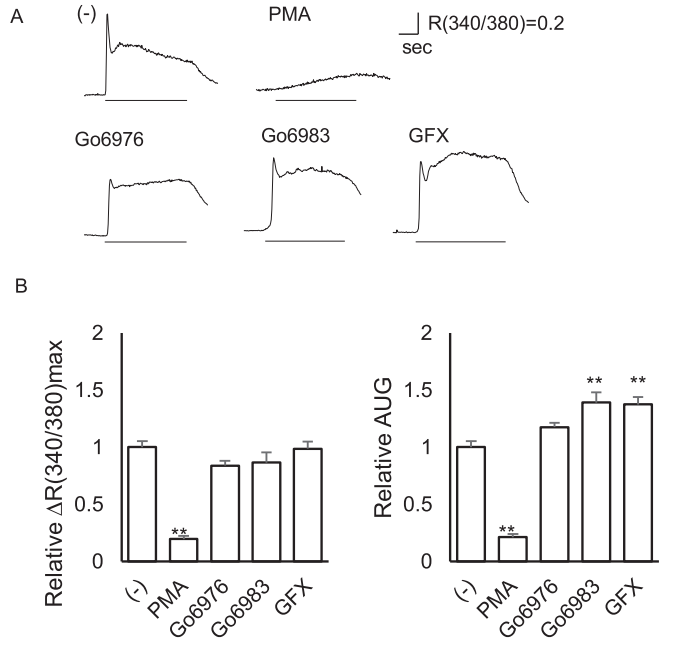

Fig. 2. The effects of pharmacological modifications of PKC on the glutamate-induced sustained calcium increases in astrocytes cultured without growth factors. (A) Representative calcium increases. Cells were pretreated with 300 nM PMA or 1 μM PKC inhibitors (Go6972, Go6983 or GFX) and then stimulated with 30 μM glutamate, as indicated by underlines. (B) Quantitative comparisons of calcium increases by relative peak amplitude; ΔR(340/380)max and relative area under the graph; AUG. Mean ± S.E.M. **p < 0.01, Tukey's multiple comparison method with control (n = 28 cells).

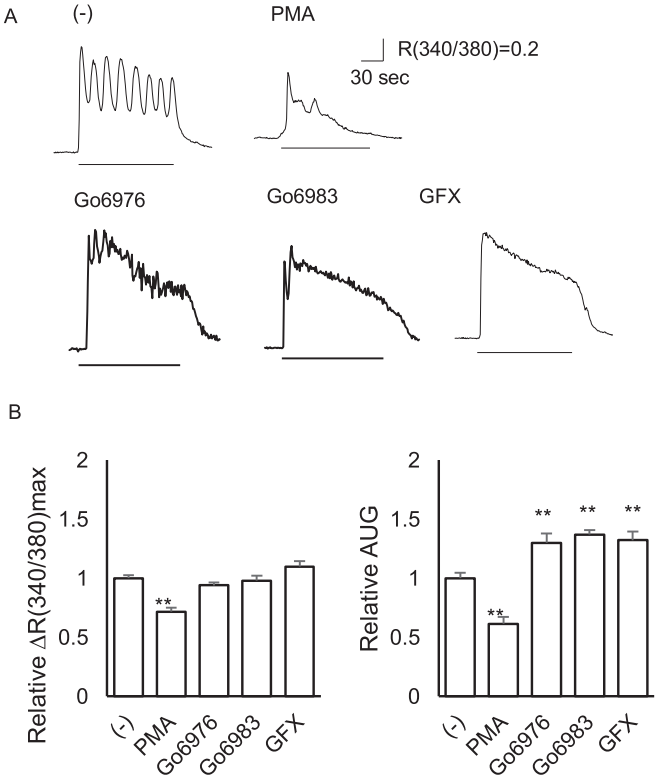

Fig. 3. The effects of pharmacological modifications of PKC on the histamine-induced calcium oscillations in astrocytes cultured with growth factors. (A) Representative calcium increases. Cells were pretreated with 300 nM PMA or 1 μM PKC inhibitors (Go6972, Go6983 or GFX) and then stimulated with 30 μM histamine, as indicated by underlines. (B) Quantitative comparisons of calcium increases by relative peak amplitude; ΔR(340/380)max and relative area under the graph; AUG. Mean ± S.E.M. **p < 0.01, Tukey's multiple comparison method with control (n = 28 cells).

activation by PMA significantly reduced both of the peak amplitudes and the area under the graphs (AUG), representing the integral of the calcium increases by 62% and 76%, respectively. A selective inhibitor of calcium dependent PKC isoforms (cPKC), Go6976, was used at 10, 100, and 1000 nM, and significantly increased the AUG by 20%, 27% and 39%, respectively, without affecting the peak amplitudes. Broad-spectrum PKC inhibitors, Go6983 and GFX, increased the AUG by 47% and 65%, respectively, without affecting the peak amplitudes at 1000 nM but not affecting them at 10 and 100 nM. These results indicate that PKC is activated during the glutamate stimulations and suppresses the calcium increases, especially in the falling phases during the repetitive calcium increases.

3.2. Glutamate-induced non-oscillatory calcium increase

The involvement of PKC in non-oscillatory calcium increases in astrocytes cultured without growth factors was examined pharmacologically. As shown in Fig. 2, PMA significantly reduced both the peak amplitudes and the AUG of the calcium increases by 80% and 79%, respectively. 1 μM Go6976 did not affect the calcium increase, whereas Go6983 and GFX at the same concentration significantly increased the AUG by 39% and 37%, respectively, without affecting the peak amplitudes. These results indicate that PKC suppressed the glutamate-induced non-oscillatory calcium increases in a similar manner to the oscillatory calcium increases, but not involving cPKC.

3.3. Histamine-induced calcium oscillation

To address the stimulation specificity of the involvement of PKC in the calcium oscillation, the histamine-induced calcium oscillations in the astrocytes cultured with the growth factor have been examined pharmacologically. As shown in Fig. 3, PMA significantly reduced both the peak amplitudes and the AUG of the calcium increases by 28% and 40%, respectively. Go6976, Go6983, and GFX increased the AUG by 30, 37, and 32%, respectively, without affecting peak amplitudes. These results indicate that PKC is similarly involved in the histamine-induced calcium oscillations in astrocytes.

4. Discussion

To address the involvement of PKC in the calcium dynamics in astrocytes, the present study examined the effects of the pharmacological modifications of PKC on glutamate- or histamine-induced calcium releases in astrocytes cultured in conditions preferentially up-regulate or down-regulate of the calcium oscillations. The PKC activation by PMA largely reduced the peak amplitudes and the AUG of both oscillatory and non-oscillatory calcium releases. Furthermore, the effect of PMA was consistent between the glutamate- and histamine-induced calcium oscillations. Thus, the activation of PKC presumably leads to the suppression of the cellular machineries involved in the calcium releases. PKC phosphorylates phospholipase Cβ (PLCβ) and reduces the inositol phospholipid hydrolysis via G protein signaling by blocking the coupling between PLCβ and G protein (16,17). PKC also phosphorylates the type 2 inositol 1,4,5 trisphosphate (IP_3) receptor, which is the dominant subtype in astrocytes, and reduces the affinity to IP_3 (18,19). Furthermore, PKC phosphorylates and desensitizes mGluR5, which

mediates the glutamate-induced calcium releases in astrocytes, and the histamine H1 receptor (20,21). Since each of these suppressions is less than a 50% reduction, the robust inhibitory effect of PMA is assumed to reflect the synergistic influence of these inhibitions.

In our previous studies we have quantified the number of peaks during calcium increases and determined the influence of growth factors and cytokines, or agonist concentrations, on calcium oscillations (7,15). Although the same quantification method was used in the present study to determine the concentration-dependent effects of PKC inhibitors on the calcium oscillation in astrocytes, clear results were not obtained. The PKC inhibitors did not simply reduce the number of peaks, they also increased the variance. The conditions that we used to detect the peaks significantly affected the results of our quantification. Thus, the present study quantified the pharmacological effects by measuring the peak amplitude and the AUG of the calcium increases, and interpreted the results by focusing on the removal of cytosolic calcium.

The broad-spectrum PKC inhibitors, Go6983 and GFX increased the AUG of oscillatory and non-oscillatory calcium increases without affecting the peak amplitudes. The calcium oscillations were converted to non-oscillatory sustained calcium increases, due to the increase of calcium concentration between peaks. Thus, two inhibitors most likely abolished the falling phase of the repetitive calcium increases by inhibiting the removal of cytosolic calcium. It has been demonstrated that the excretion of cytosolic calcium by plasma membrane calcium ATPase is activated by PKC following inositol phospholipid hydrolysis, and plays a crucial role in reducing cytosolic calcium to the basal level in neurons (22). Na^+/Ca^{2+} exchanger 1, which is a dominant subtype in astrocytes, is also activated by PKC (23,24). Our previous study has demonstrated that the calcium oscillations of astrocytes cultured in a defined medium containing the growth factors persist even in the absence of extracellular calcium (7). Consequently, a large portion of the cytosolic calcium is likely to be absorbed into the endoplasmic reticulum by SERCA to maintain the repeated calcium releases in the astrocytes. Thus, it is suggested that the PKC inhibitors increases the AUG of the oscillatory and non-oscillatory calcium releases by inhibiting the PKC-mediated SERCA activation. Although PKC has been shown to up-regulate the SERCA-mediated calcium absorption in neurons, it is still to be determined if PKC activates SERCA by direct phosphorylation or by phosphorylating proteins regulating SERCA (25). As shown in our previous study, the increase of the SERCA expression underlies the up-regulation of the astrocyte calcium oscillation by the growth factors (10), thus it is suggested that the SERCA activity is maintained to an extent that is sufficient for the calcium oscillations, not only by gene expression but also by the PKC-mediated activation following inositol phospholipid hydrolysis. The present results do not exclude the possibility that the PKC inhibitors suppress the phosphorylation of the receptors and calcium release machineries, which are also targets of PKC, as mentioned above, and increase the AUG. However, if this is the case, then the peak amplitudes are more likely to be affected.

The cPKC selective inhibitor, Go6976, increased the AUG of the glutamate-induced calcium oscillations, even at 10 nM, which is 100 times lower than the effective concentration of broad PKC inhibitors, whereas it did not affect the non-oscillatory calcium increases. The selective effect of Go6976 on calcium oscillations suggests that cPKC is more effectively activated during calcium oscillation in astrocytes, as previously reported in other cell types (26). The up-regulation of cPKC isoforms in astrocytes by the growth factors may underlie the selective effect of Go6976. The effect of Go6976 at lower concentrations may reflect the involvement of PKCμ, which Go6976 inhibits more potently than Go6893 and GFX (27). It has been reported that PKCμ, which is also designated as PKD, is activated during receptor stimulation; leading to inositol phospholipid hydrolysis in astrocytes (28). Further analysis on the expressions of PKC isoforms in astrocytes, with or without growth factor treatments, will address the unique effects of Go6976.

The calcium increases were suppressed by PMA but enhanced by broad-spectrum PKC inhibitors. This suggests that the receptor stimulations to induce inositol phospholipid hydrolysis and calcium releases activates PKC to an extent which is smaller than the activation by PMA. The present result suggests that this intermediate activation of PKC influences both oscillatory and non-oscillatory calcium increases in the same manner. Thus, it is considered that the growth factors are less likely to regulate calcium oscillation by altering PKC pathways. However, it is still possible to assume that the growth factors alter the expression of PKC isoforms, especially cPKC, and modify the PKC pathways for generating the calcium oscillation. If the growth factors make the intermediate activation of PKC more calcium dependent by increasing the expression of cPKC, then the calcium absorption by SERCA, which is likely to be enhanced by PKC (as discussed above), will also become more calcium dependent and will preferentially contribute to calcium oscillation. The assumed contribution of the intermediate activation of PKC to astrocyte calcium dynamics are summarized in Fig. 4. The growth factors increases SERCA on calcium store and enlarge store size (1,2). Glutamate stimulation (3) induces calcium release from IP_3R2 (4,5) and partially activates

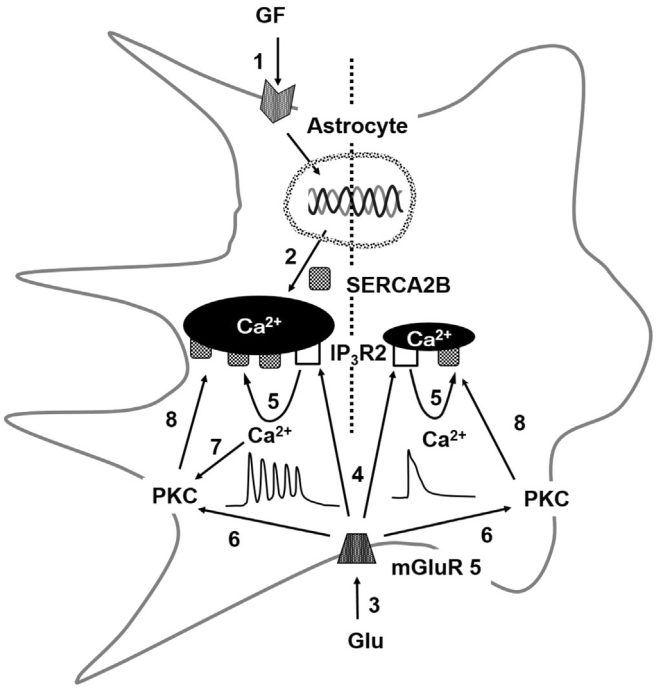

Fig. 4. PKC in astrocyte calcium dynamics. The left half represents the glutamate-induced calcium oscillation in astrocytes cultured in the presence of growth factors, whereas the right half represents the glutamate-induced non-oscillatory calcium increases in an astrocyte cultured without growth factors. The growth factors (GF) (1) up-regulates astrocyte calcium oscillation by increasing SERCA2B expression (2) and calcium store size. Glutamate stimulation (3) induces IP_3 production via mGluR5 (4). IP_3 induces calcium release from Type 2 IP_3 receptor (IP3R2) and the calcium is reabsorbed by SERCA2B (5). mGluR5 also increases diacylglycerol production, which activates PKC (6). In the GF-treated astrocytes, intracellular calcium is also involved in PKC activation (7). PKC enhances SERCA-mediated removal of cytosolic calcium (8). The PKC activator, PMA, up-regulates the process represented by (8). The broad-spectrum PKC inhibitors, Go6983 and GFX, block the process represented by (8), whereas the cPKC inhibitor, Go6976, blocks the PKC isoforms activated synergistically by the processes represented by (6) and (7).

PKC (6). In growth factor treated cells, calcium release enhances PKC activation (7), which positively regulates the calcium uptake by SERCA2B (8). Calcium oscillation in growth factor treated astrocytes is likely attributed to the synergistic effect of the calcium dependent SERCA activation via PKC and increased capacity of the calcium uptake. Further analysis of the expression of PKC isoforms and isoform selective ablation by siRNA or genome editing will address this issue. The present pharmacological data demonstrate that the calcium dynamics in astrocytes are under the influence of PKC and that PKC is an effective means to control the spatiotemporal pattern of the calcium increases in astrocytes.

Conflict of interest

None declared.

Acknowledgment

We thank Ms. Hiromi Yanaka for her excellent secretarial assistance. We thank Ms. Ikuko Ishikawa and Ms. Tomoko Morimoto for their technical assistance. This work was supported by Grant-in Aid for Scientific Research on Priority Areas (B) (10214204), Grant-in Aid for Scientific Research on Priority Areas (15082101), Grant-In-Aid For Scientific Research On The Innovative Area (23122513), Grant-in-Aid for Scientific Research (B) (23300133, Japan), all from the Ministry of Education, Science and Culture in Japan. And also supported by Smoking Research Foundation (Japan), Takeda Science Foundation (Japan), Centers of Biomedical Research Excellence (PR15636) from National Institute of Health/National Center for Research Resources (USA).

References

(1) Volterra A, Liaudet N, Savtchouk I. Astrocyte Ca(2)(+) signalling: an unexpected complexity. Nat Rev Neurosci. 2014;15:327–335.
(2) Di Castro MA, Chuquet J, Liaudet N, Bhaukaurally K, Santello M, Bouvier D, et al. Local Ca2+ detection and modulation of synaptic release by astrocytes. Nat Neurosci. 2011;14:1276–1284.
(3) Shigetomi E, Tong X, Kwan KY, Corey DP, Khakh BS. TRPA1 channels regulate astrocyte resting calcium and inhibitory synapse efficacy through GAT-3. Nat Neurosci. 2012;15:70–80.
(4) Gordon GR, Choi HB, Rungta RL, Ellis-Davies GC, MacVicar BA. Brain metabolism dictates the polarity of astrocyte control over arterioles. Nature. 2008;456:745–749.
(5) Pascual O, Casper KB, Kubera C, Zhang J, Revilla-Sanchez R, Sul JY, et al. Astrocytic purinergic signaling coordinates synaptic networks. Science. 2005;310:113–116.
(6) Morel L, Higashimori H, Tolman M, Yang Y. VGluT1+ neuronal glutamatergic signaling regulates postnatal developmental maturation of cortical protoplasmic astroglia. J Neurosci. 2014;34:10950–10962.
(7) Morita M, Higuchi C, Moto T, Kozuka N, Susuki J, Itofusa R, et al. Dual regulation of calcium oscillation in astrocytes by growth factors and pro-inflammatory cytokines via the mitogen-activated protein kinase cascade. J Neurosci. 2003;23:10944–10952.
(8) Ding S, Fellin T, Zhu Y, Lee SY, Auberson YP, Meaney DF, et al. Enhanced astrocytic Ca2+ signals contribute to neuronal excitotoxicity after status epilepticus. J Neurosci. 2007;27:10674–10684.
(9) Delekate A, Fuchtemeier M, Schumacher T, Ulbrich C, Foddis M, Petzold GC. Metabotropic P2Y1 receptor signalling mediates astrocytic hyperactivity in vivo in an Alzheimer's disease mouse model. Nat Commun. 2014;5:5422.
(10) Morita M, Kudo Y. Growth factors upregulate astrocyte [Ca2+]i oscillation by increasing SERCA2b expression. Glia. 2010;58:1988–1995.
(11) Bradley SJ, Challiss RA. Defining protein kinase/phosphatase isoenzymic regulation of mGlu(5) receptor-stimulated phospholipase C and Ca(2)(+) responses in astrocytes. Br J Pharmacol. 2011;164:755–771.
(12) Devaraju P, Sun MY, Myers TL, Lauderdale K, Fiacco TA. Astrocytic group I mGluR-dependent potentiation of astrocytic glutamate and potassium uptake. J Neurophysiol. 2013;109:2404–2414.
(13) Steinberg SF. Structural basis of protein Kinase C isoform function. 2008. p. 1341–1378. 2008-10-01 00:00:00.
(14) Morita M, Kozuka N, Itofusa R, Yukawa M, Kudo Y. Autocrine activation of EGF receptor promotes oscillation of glutamate-induced calcium increase in astrocytes cultured in rat cerebral cortex. J Neurochem. 2005;95:871–879.
(15) Morita M, Susuki J, Moto T, Higuchi C, Kudo Y. A novel method to quantify calcium response pattern and oscillation using fura2 and acridine orange. J Pharmacol Sci. 2004;94:25–30.
(16) Ryu SH, Kim UH, Wahl MI, Brown AB, Carpenter G, Huang KP, et al. Feedback regulation of phospholipase C-beta by protein kinase C. J Biol Chem. 1990;265:17941–17945.
(17) Cunningham ML, Filtz TM, Harden TK. Protein kinase C-promoted inhibition of Galpha(11)-stimulated phospholipase C-beta activity. Mol Pharmacol. 1999;56:265–271.
(18) Arguin G, Regimbald-Dumas Y, Fregeau MO, Caron AZ, Guillemette G. Protein kinase C phosphorylates the inositol 1,4,5-trisphosphate receptor type 2 and decreases the mobilization of Ca2+in pancreatoma AR4-2J cells. J Endocrinol. 2007;192:659–668.
(19) Petravicz J, Boyt KM, McCarthy KD. Astrocyte IP3R2-dependent Ca(2+) signaling is not a major modulator of neuronal pathways governing behavior. Front Behav Neurosci. 2014;8:384.
(20) Uchino M, Sakai N, Kashiwagi K, Shirai Y, Shinohara Y, Hirose K, et al. Isoform-specific phosphorylation of metabotropic glutamate receptor 5 by protein kinase C (PKC) blocks Ca2+ oscillation and oscillatory translocation of Ca2+-dependent PKC. J Biol Chem. 2004;279:2254–2261.
(21) Chen X, Egly C, Riley AM, Li W, Tewson P, Hughes TE, et al. PKC-dependent phosphorylation of the H1 histamine receptor modulates TRPC6 activity. Cells. 2014;3:247–257.
(22) Usachev YM, DeMarco SJ, Campbell C, Strehler EE, Thayer SA. Bradykinin and ATP accelerate Ca(2+) efflux from rat sensory neurons via protein kinase C and the plasma membrane Ca(2+) pump isoform 4. Neuron. 2002;33:113–122.
(23) He S, Ruknudin A, Bambrick LL, Lederer WJ, Schulze DH. Isoform-specific regulation of the Na+/Ca2+ exchanger in rat astrocytes and neurons by PKA. J Neurosci. 1998;18:4833–4841.
(24) Iwamoto T, Pan Y, Nakamura TY, Wakabayashi S, Shigekawa M. Protein kinase C-dependent regulation of Na+/Ca2+ exchanger isoforms NCX1 and NCX3 does not require their direct phosphorylation. Biochemistry. 1998;37:17230–17238.
(25) Usachev YM, Marsh AJ, Johanns TM, Lemke MM, Thayer SA. Activation of protein kinase C in sensory neurons accelerates Ca2+ uptake into the endoplasmic reticulum. J Neurosci. 2006;26:311–318.
(26) Gonzalez-Garcia JR, Machaty Z, Lai FA, Swann K. The dynamics of PKC-induced phosphorylation triggered by Ca2+ oscillations in mouse eggs. J Cell Physiol. 2013;228:110–119.
(27) Gschwendt M, Dieterich S, Rennecke J, Kittstein W, Mueller HJ, Johannes FJ. Inhibition of protein kinase C mu by various inhibitors. Differentiation from protein kinase C isoenzymes. FEBS Lett. 1996;392:77–80.
(28) Carrasquero LM, Delicado EG, Sanchez-Ruiloba L, Iglesias T, Miras-Portugal MT. Mechanisms of protein kinase D activation in response to P2Y(2) and P2X7 receptors in primary astrocytes. Glia. 2010;58:984–995.